# Toxicity of the extracts of sisal waste, obtained from decortications of the *Agave sisalana*

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***Abstract*—** *Agave sisalana (sisal) is a monocotyledonous plant of great economic interest because it is a source of hard fiber in semi-arid areas. It has also been widely used by rural smallholders for animal feed in several countries. The toxic effects of plant on the animal reproduction are unknown. Then, the study investigated the possible side effects of different extracts derived from the processing of sisal leaves on the reproductive organs weight and testicular tissue of adult rats. The animals were treated with the extract obtained by acid hydrolysis (100 mg/kg body weight– b.w.; EHA/100), dry precipitate extract (250 and 500 mg/kg b.w.; EPS/250 and EPS/500), hexane extract (50 and 100 mg/kg b.w.; HEX/50 and HEX/100) or distilled water (Control group). Treatments were performed daily for 30 consecutive days, oral route (gavage) a single time daily. The results showed that in the group treated with EHA/100 extract only the weight of the seminal vesicles has been changed, but in the EPS/250, EPS/500, HEX/50 and HEX/100 groups there was significant increase ($p<0.05$) in the testes, epididymides and seminal glands weight, in comparison to the control group. All groups treated with the different extracts showed histopathological changes in the testes, characterized mainly by depletion of seminiferous epithelium, detachment of immature germ cells, scarcity sperm in the tubular lumen and interstitial hemorrhage, which occurred in a dose-independent manner in EPS and HEX groups. In conclusion, the different extracts of A. sisalana changed the reproductive organs weight and were promoters from gonadotoxic effect in rats.*

***Keywords*— *steroidal saponins, reproduction, testicular tissue.***

## I. INTRODUCTION

*Agave sisalana* Perrine (sisal) is a monocotyledonous plant of great economic interest because it is a source of hard fiber in semi-arid areas. Brazil is the world's largest producer and exporter of sisal fibers. Only 4% of the decortications of the sisal leaves produce fiber, and the remaining material (waste) is commonly discarded by sisal farms (Martin et al., 2009).

The sisal waste consists of water, parenchymatous tissue, cellulose, fibers of various sizes, inorganic compounds and components related to primary and secondary metabolism. This waste material is rarely used, despite its indication for use as an organic fertilizer, a supplement in ruminant feed (Nery et al., 2009) and a raw material for the production of medicine (Kerboeuf et al., 2008, Botura et al, 2013).

Previous studies reported that *A. sisalana* had several biological effects, including antimicrobial, anti-inflammatory and anthelmintic properties (Pereira da Silva et al., 2002; Ohtsuki et al., 2004; Pereira da Silva et al., 2006; Zhang et al., 2008; Mimaki, 2009; Qin et al., 2012; Li et al., 2010; Kang et al., 2012). Steroidal saponins (Pérez, et al., 2013) and flavonoids (Chen et al., 2009) are among the secondary metabolites that have been isolated from this plant. However, few studies reporting toxicity of these metabolites have been described, concerning usually the triterpenoid saponins (Chen et al., 2011), and the real benefits and risks of *A. sisalana* should yet be evaluate.

Then, the present study investigated the toxicity of the extracts of *A. sisalana* waste, obtained from decortications, on the reproductive organs weight and testicular tissue of adult rats.

# II. MATERIAL AND METHODS

## 2.1 Animals

Male Wistar rats (*Rattus norvegicus*), 12 weeks old, weighing approximately 300 g, were kept in appropriate cage at the Central Biotherium from the Universidade Estadual Paulista (UNESP - Assis, Brazil) in room under controlled temperature and luminosity (22ºC ± 2ºC; 12-h light/dark photoperiod, respectively). Commercial diet (Nuvilab CR-1™, Colombo, PR, Brazil) and water were offered *ad libitum*. The experimental protocol followed the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation and was approved by the Ethical Committee for Animals Use (Permit number: 025/2010 and 010/2012).

## 2.2 Obtaining sisal extracts

The different types of *A. sisalana* extracts were obtained from the Pharmacology and Phytotherapy Laboratory of the Faculty of Sciences and Letters (UNESP – Assis, Brazil).

## 2.3 Experimental Groups

The animals were weighed and randomly divided into six (n = 5 animals/group), as follow: a) Control (0.5 mL distilled water); b) treatment with the extract obtained by acid hydrolysis at a concentration of 100 mg/kg b.w. (EHA/100); c) treatment with dry precipitate extract at a concentration of 250 mg/kg b.w. (EPS/250); d) treatment with dry precipitate extract at a concentration of 500 mg/kg b.w. (EPS/500); e) treatment with hexane extract at a concentration of 50 mg/kg b.w (HEX/50), and f) treatment with hexane extract at a concentration of 100 mg/kg b.w. (HEX/100). Only the extract obtained by acid hydrolysis was administered to the animals in a single concentration.

The treatments were performed during 30 consecutive days, orally (gavage), a single time daily (7:00 h a.m).

## 2.4 Euthanasia of animals and tissue preparation

Following the experimental period, the animals were weighed and euthanized with an overdose of the anaesthetic sodium thiopental (Thiopentax™, Cristalia, São Paulo, Brazil), via intraperitoneal injection. The reproductive organs (testes, epididymides, seminal glands and prostate) were collected and weighed. The relative weight of each organ (organ weight/body weight x 100; expressed as g/100g) was obtained.

The testes were fixed in Bouin's solution, dehydrated in ethanol solutions and clarified in xylene. In sequence, the material was embedded in paraffin (Paraplast, Oxford-Labware, St. Louis, USA) and the blocks were sliced into 5µm-thick sections and then stained with haematoxylin and eosin (HE). Finally, the slides were analyzed and images captured with a digital photomicroscope (Scope A1-Axio coupled with video camera AxioCam ICc3 and digitalized by the software Axio Vision, version 4.7.2; Carl Zeiss, Jena, Germany).

## 2.5 Statistical analysis

The data were analyzed by parametric analysis of variance ANOVA, complemented by Tukey's test, and the results were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using Bioestat software, version 5.0. Statistical significance was set at $P < 0.05$.

# III. RESULTS

## 3.1 Reproductive organs weight

Table 1 shows that there was no significant effect ($P>0.05$) of treatment with the acid hydrolysis extract (EHA/100) on the testes, epididymides and prostate weight, but there was difference ($P<0.05$) on the full and empty seminal glands weight, comparatively to the control group.

The administration of the dry precipitate extract at the concentrations of 250 and 500 mg/kg (EPS/250 and EPS/500 groups), promoted an increase significant on the testes, epididymides and empty seminal glands weight of animals. The prostate

weight was similar (P>0.05) in the EPS/250 and control groups. At the concentration of 500 mg/kg (EPS/500), the full seminal glands weight was similar to the control group, but the prostate weight was decreased. The groups treated with EPS did not differ significantly between them.

The hexane extract, at the concentrations of 50 mg/kg (HEX/50) and 100 mg/kg (HEX/100), increased significantly the epididymides and empty seminal glands weight, in comparison to the control group. The HEX/100 group presented an increase (P<0.05) on the testes weight in comparison to the control and HEX/50 groups. Only the concentration lower of hexane extract (HEX/50 group) promoted an increase on the full seminal glands and prostate, comparatively to the control group.

**TABLE 1**
**REPRODUCTIVE ORGANS WEIGHT, IN g/100 g b.w., IN THE CONTROL GROUP AND GROUPS TREATED WITH DIFFERENT EXTRACTS OF *A. SISALANA* (ACID HYDROLYSIS AT A CONCENTRATION OF 100 mg/kg - EHA/100; DRY PRECIPITATE AT A CONCENTRATION OF 250 AND 500 mg/kg - EPS/250 AND EPS/500; HEXANE FRACTION AT A CONCENTRATION OF 50 AND 100 mg/kg - HEX/50 AND HEX/100).**

| Groups | Testes | Epididymides | Full seminal glands | Empty seminal glands | Prostate |
|---|---|---|---|---|---|
| Control | 0.70 ± 0.07a | 0.30 ± 0.04a | 0.28 ± 0.10a | 0.13 ± 0.04a | 0.20 ± 0.03a |
| EHA/100 | 0.80 ± 0.09a | 0.32 ± 0.04a | 0.50 ± 0.04b | 0.27 ± 0.04b | 0.20 ± 0.03a |
| EPS/250 | 0.88 ± 0.11b | 0.42 ± 0.08 b | 0.40 ± 0.06b | 0.18 ± 0.03b | 0.19 ± 0.03ab |
| EPS/500 | 0.94 ± 0.08b | 0.41 ± 0.09 b | 0.38 ± 0.05ab | 0.20 ± 0.04b | 0.14 ± 0.03b |
| HEX/50 | 0.76 ± 0.04a | 0.44 ± 0.14b | 0.51 ± 0.09b | 0.31 ± 0.07b | 0.25 ± 0.03b |
| HEX/100 | 0.98 ± 0.03b | 0.52 ± 0.15b | 0.41 ± 0.10a | 0.23 ± 0.06b | 0.21 ± 0.02ab |

**Data are expressed as the mean ± SD. In the same column, values followed by different letters indicate statistical differences between the groups (P < 0.05). N = 5 animals/group.**

### 3.2 Analysis of testicular tissue

In the animals of control group (Figure 1A), the seminiferous tubules presented morphological characteristics well defined, with epithelium constituted by multiple layers of germ cells topographically organized, represented by peripheral spermatogonia, intermediate spermatocyte and spermatid in adluminal position. In the tubular lumen, considerable quantity of spermatozoa was present. The interstitial compartment constituted by loose connective tissue, presented blood and lymphatic vessels, nerves and Leydig cells.

In the group treated with EHA/100 (Figures 1B-D), several region of testes showed atrophied seminiferous tubules and/or with epithelial depletion, characterized by few layers of germ cells. The seminiferous epithelium presented vacuoles of varied size and degenerative cells, which showed intense acidophilia and pyknotic nucleus. In the tubular lumen, scarce amount of gametes was present, or amorphous mass and immature germ cells occupied inside. The tubular interstitium and the Leydig cells showed normal morphological aspect.

In the group treated with dry precipitate extract, at the concentrations of 250 mg/kg (Figures 1E, 1F) and 500 mg/kg (Figures 1G, 1H), there was rupture of the seminiferous tubular structure, which showed disorganization of the epithelial cytoarchitecture (Figures 1E, 1G) and desquamation of germ cells, that resulted in several immature cells in the tubular lumen (Figures 1E, 1H). Scant amount of gametes was present in lumen from most seminiferous tubules. In the interstitial tissue was observed vascular congestion and areas of hemorrhagic aspect (Figures 1F, 1G), in addition to Leydig cells apparently atrophied.

The rats treated with hexane extract, at the concentrations of 50 mg/kg (Figure 1I) and 100 mg/kg (Figures 1J, 1K), exhibited seminiferous tubules with scant amount of gametes into lumen and there was detachment of germ cells layers in the epithelium. In both groups, the tubular interstitium showed hemorrhagic and congests areas.

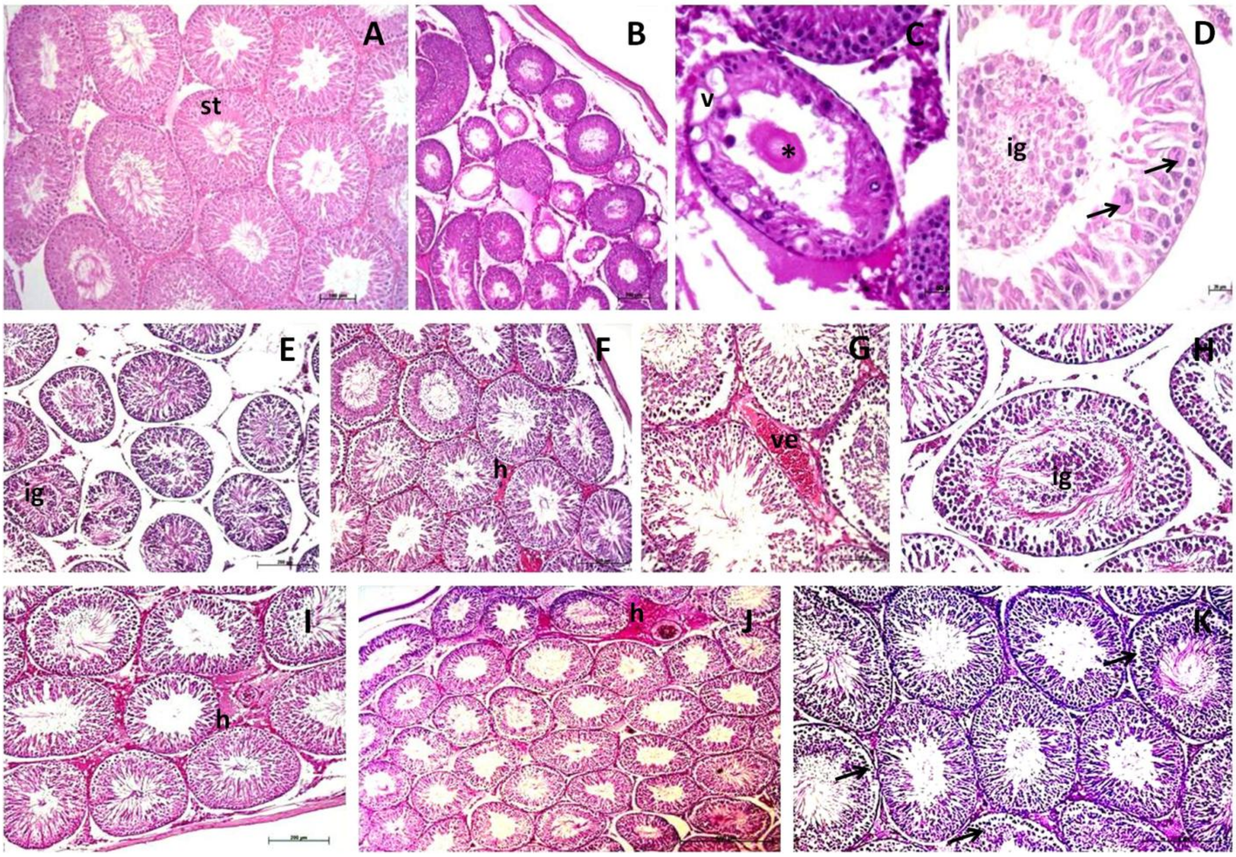


**FIGURE 1 – PHOTOMICROGRAPHS OF THE RAT TESTIS IN DIFFERENT EXPERIMENTAL GROUPS. (A) Control group, showing the morphological integrity of the seminiferous tubules (st). (B-D) EHA/100 group, showing seminiferous tubules atrophied, with epithelial depletion and vacuolization (v), with germ cells in degeneration (arrows) and with amorphous mass (asterisk) or immature germ cells (ig) in the tubular lumen. (E, F) EPS/250 group, showing epithelial disorganization, immature germ cells (ig) in the tubular lumen and hemorrhagic areas (h) in the interstitial tissue. (G, H) EPS/500 group, showing blood vessel congest (ve), lack of morphological integrity of the seminiferous epithelium and immature germ cells (ig) in the tubular lumen. (I) HEX/50 group, highlighting hemorrhagic areas (h) in the interstitium. (J, K) HEX/100 group, showing paucity of spermatozoa in the tubular lumen, detachment of germ cells layers of epithelial wall (arrows) and hemorrhagic area (h) in the interstitial tissue. HE.**

## IV. DISCUSSION

*A. sisalana* has pharmacological properties of interest due to the main phytochemicals and in the popular medicine is used for the treatment of skin diseases, syphilis, liver disease, tuberculosis and jaundice. The plant is rich in steroidal saponins and also contains flavonoids and homoisoflavonoids (Ding et al., 1989; Botura, 2010), which presents antibacterial, antifungal, antihelmintic and anti-inflammatory activities (Ujikawa and Purchio, 1989; Kassu et al., 1999; Pizarro et al., 1999; Santos et al., 2009). Several studies have documented the different pharmacological properties of *A. sisalana*, but its side effects on reproduction are unknown and deserve further attention.

In animal experiments, there are clinical signs that may be indicative of toxicity promoted by the administered agent. These signs include changes in behavior, ataxia, salivation, vomiting, diarrhea, polyphagia, fever, weakness, tremors, convulsions and death (Dallegrave and Sebben, 2008). In the present study, the different concentrations and types of *A. sisalana* extract did not promote signs of toxicity in animals during the experimental period. Similarly, Dunder et al. (2013) observed that there was lack of toxicity of the hexane fraction of *A. sisalana*, when administered in an acute dose to rats and mice at concentrations of 5, 10, 25 and 50 mg/kg. Our study showed that high doses of the hexane fraction of *A. sisalana* (50 and 100 mg / kg), administered by chronic treatment were safe for the animals, as well as other types of plant extracts. Previous study (Qin et al., 2009) evaluated the potential toxicity of the steroidal saponins of *Dioscorea zingiberensis* in mice and verified that only at high doses the animals showed signs of toxicity, such as asthenia, piloerection, anorexia, syncope and weight loss.

In the evaluation of the reproductive toxicity of the medicinal plant has been observed that EPS and HEX extracts promoted alterations in the weight of testis, epididymis, seminal glands and prostate in a dose-independent manner, while EHA extract administered at one concentration only affect seminal glands weight. The increase in testicular weight in the EPS/250, EPS/500 and HEX/100 groups was probably due to the histological changes observed in the organ, such as the accumulation of immature germ cells into the lumen of several seminiferous tubules and hemorrhage into the tubular interstitium. In the animals of HEX/50 group, the changes in the testis histoarchitecture were less pronounced and do not reflect a significant change in gonad weight. In EHA/100 group, the morphological damage in gonadal structure was insufficient to promote any change in organ weight. Although in this study was not carried out a histopathological evaluation of the genital duct and sex glands, the changes observed in the weight of these organs in EPS and HEX groups indicates that these extracts of *A. sisalana* may have concentrations of its chemical constituents with deleterious effects on organ morphology. This result should be further investigated in order to assess the possibility of epithelial hyperplasia and/or hypertrophy, change in the interstitial tissue constituents or changes in the amount of secretion and gametes within the lumen.

Studies on medicinal plants that may interfere with male fertility generates much interest and the reproductive tract is sensitive to the effects of various chemical components of the plant. In *A. sisalana*, the steroidal saponins are the main components responsible for the biological activity of the juice extracted from the plant (Ding et al., 1989) and this activity depends on the concentration and composition of active saponins (Dinchev et al., 2008). The isolation and structural knowledge of the saponins present in *A. sisalana* (Chen et al, 2011), *A. macroacantha* (Eskander et al., 2010) and *A. utahensis* (Yokosuka and Mimaki, 2009) were extensively evaluated. According to Francis et al. (2002), the effects of saponins appear to be related to interactions with steroid receptors since the basic structures of saponins are similar to steroidal hormones, important for reproductive function. Cytotoxic effects of steroidal saponins were reported in breast and lung cancer cell lineage (Chen et al, 2011).

In this study, different types of *A. sisalana* extracts promoted histopathological changes in the testes of the animals, characterized by depletion of the seminiferous epithelium, detachment of immature germ cells, intraepithelial vacuolization, interstitial hemorrhage, vascular congestion and paucity of spermatozoa in the tubular lumen. According to Qin et al. (2009), any extract containing saponins has the potential to cause hemolysis of red blood cells. In their study, rats treated with high doses of saponins derived of *Dioscorea zingiberensis* (1125, 2250, 4500 and 9000 mg/kg), showed in the liver tissue a regular presence of cytoplasmatic vacuoles, vascular congestion accompanied by hemorrhages and sporadic focal necrosis, and atrophy of organ. This report corroborates the results observed in testis tissue of rats, indicating that the male gonad is a target organ of the effects of steroidal saponins present in *A. sisalana*. In other study (Oliveira et al., 2015), rats supplemented with extracts and fractions of *Tribulus terrestris* fruits, a plant rich in steroidal saponins, presented changes in the testicular biometric and morphometric parameters, and there was no improvement in semen quality.

In conclusion, the extracts EHA, EPS and HEX, derived from *A. sisalana* waste, affected the reproductive organs weight and were promoters from gonadotoxic effect in rats, indicating that can affect the fertility of animals. Further studies are needed to evaluate the occurrence of injury in fertility and pregnant outcome in animals treated with *A. sisalana*.

## CONFLICT OF INTEREST

The authors declare that has no conflict of interest.

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